

Paragraph bridging pages 19-20

cDNA was made from 250 ng of the isolated poly A⁺ RNA using SMART™ PCR cDNA Library Construction kit (Clontech) according to the protocol recommended by the manufacturer. In brief, 1st strand cDNA was made by combining 250 ng A⁺ RNA with 10 pmol SMART oligonucleotide (5'-TACGGCTGCGAG AAGACGACAGAAGGG-3') (SEQ ID NO:5) and 10 pmol CDS/3' PCR primer (Oligo(dT)30 N-1N (N=A, G, C, or T; N-1 = A, G, C or T)) in a final volume of 5 µl, and incubated at 72°C for 2 min and then placed directly on ice for 2 min to denature the RNA. Then enzyme and buffer were added to the reaction mixture to a final volume of 10 µl, consisting of 50 mM Tris/HCl, pH 8.3, 6mM MgCl₂, 75mM KCl, 2mM DTT, 1mM dATP, dCTP, dGTP and dTTP respectively and 200 U SuperScript™ II reverse transcriptase (Gibco BRL), and then incubated at 42°C for 1h. Synthesis of 2nd strand was done by PCR in a final volume of 100 µl, containing 2 µl of the 1st strand reaction as template, 40 mM Tricine/KOH pH 9:2 (25°C), 15 mM KOAc, 3.5 mM Mg(OAc)₂, 3.75 µg/ml BSA, 0.2mM of dATP, dCTP, dGTP and dTTP respectively, 1U Advantage cDNA Polymerase Mix (Clontech), 0.2 µM 5'-PCR primer (5'-TACGGCTCCGAGAAGACGACAGAA-3') (SEQ ID NO:6) and CDS/3'-PCR primer respectively.

Paragraph bridging pages 20 and 21

Degenerated oligonucleotide primers were designed from two conserved regions (GQDPYH and VFLLWG) from known UNG-amino acid sequences. Codon usage for Atlantic cod were also considered when designing the primers. The UNG fragment was generated by PCR with cod liver cDNA as template in a final volume of 50 µl, containing 10 mM Tris/HCl pH 9.0 (25°C), 50 mM KCl, 0.1% Triton X-100, 10 ng cDNA, 0.2 mM dATP, dCTP, dGTP

B³
correl.

and dTTP respectively, 2.0 μ M upstream primer (5'-GGH-CAR-GAY-CCC-TAY-CA-3') (SEQ ID NO:7) and downstream primer (5'-DCC-CCA-SAG-SAG-RAA-VAC-3')¹ (SEQ ID NO:8) respectively and 2.5 U Taq-polymerase (Promega). PCR was carried out at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min, and a final extension step of 72°C for 5 min.

Paragraph bridging pages 21 and 22

B⁴

The sequence deduced from the 300 bp fragment of the UNG-gene was used to design two primers for both 3'- and 5'- rapid amplification of cDNA ends (RACE), with a small overlap region between the two fragments generated. Both 3'- and 5'- RACE reactions were done in a volume of 50 μ l with 1 μ l of diluted cDNA with RACE-adaptors as template, 0.2 μ M internal 3'-(5'-TGTACCGACATTGATGGCTTCAAGCAT-3') (SEQ ID NO:9) or 5'-(5'-CCCATCCGCTTAGATCTCCATGTCCAG-3') (SEQ ID NO:10) RACE primers, respectively, 0.2 μ M AP1-primer (supplied by manufacturer) (5'-CCATCCTAATACGACTCACTATAGGGC-3') (SEQ ID NO:11), 40 μ M Tricine/KOH pH 9.2 (25°C), 15 mM KOAc, 3.5 mM Mg (OAc)₂, 3.75 μ g/ml BSA, 0.2 mM of each dATP, dCTP, dGTP and dTTP and 10U Advantage cDNA Polymerase Mix (Clontech). Amplification was done in a GeneAmp 9700 thermocycler (Perkin Elmer), 94°C for 30 sec followed by 5 cycles of 94°C for 5 sec and 72°C for 3 min, 5 cycles of 94°C for 5 sec and 70°C for 3 min, and 20 cycles of 94°C for 5 sec and 68°C for 3 min.

Paragraph 3 on page 22

B⁵

Examining the sequence of the 5'-RACE-fragment indicated a double sequence, difficult to read, near the 5'-end of the fragment. However at the end of the fragment only one sequence appeared,

B^S
incl.

due to a long UTR in one of the UNG-sequences but not the other. A new primer complementary to this 5'-end was designed (5'-ATGGAATTCGATTGAGATTGGCGCCTTTGG-3') (SEQ ID NO:12) and a new PCR-reaction was carried out with this, and the 5'-RACE internal primer, with the 5'-RACE fragment as template. The PCR was carried out in a final volume of 50 μ l with 10 mM Tricine/KOH pH 9.2 (25°C), 15 mM KOAc, 3.5 mM Mg(OAc)₂, 3.75 μ g/ml BSA, 0.2 mM of dATP, dCTP, dGTP and dTTP respectively, 1U Advantage cDNA Polymerase Mix (Clontech), 10ng cDNA as template and 0.2 μ M upstream and downstream primers respectively. Amplification was done in a GeneAmp 9700 thermocycler (Perkin Elmer), 94°C for 1 min. followed by 30 cycles of 94°C for 30 sec, 60°C for 1 min and 72°C for 1 min.

Paragraph 3 on page 24

B⁶

rcUNG Δ 74:UDGL77 (5'-ACCATGGAATTCCCAAAGCA ACGCCTGCA-3') (SEQ ID NO:13) and UDGEN2 (5'-GAGCTCGTCGACTTAGAGTGCCTCTCCAGTTTATAGG-3') (SEQ ID NO:14) and 10 ng cDNA as template.

Paragraph 4 on page 24

B⁷

rcUNG Δ 81:UDGL84 (5'-CCATGGAATTCTTCGGAGAGACTTGG AGAAGA-3') (SEQ ID NO:15) and UDGEN2 and 10 ng cDNA as template.

Paragraph 5 on page 24

B⁸

rcUNG Δ 74o: (5'-ATGGAATTCGCAAAGCAACGCCTGC AGGTTTCGGAGAGACTTGGCGTCGTCAG-3') (SEQ ID NO:16) and UDGEN2 and 1 ng rcUNG Δ 81o as template.

Paragraph 1 on page 25

B⁹

rcUNGΔ81o: (5'-ATGGAATTCTTCGGAGAGACTTGGCGT
CGTGAGCTGGCTGC-3') (SEQ ID NO:17) and UDGEND2 and
10 ng cDNA as template.

Paragraph 3 on page 29

B¹⁰

OP5:
5'-TCTCTCGAGAAAAGAGAGGCTGAAGCTCCCATTG
ACGATGAGGATGA-3 (SEQ ID NO: 18).

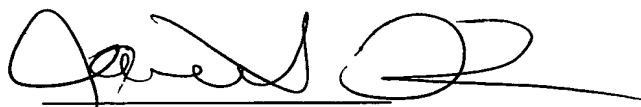
Paragraph 4 on page 29

B¹¹

NP2:
5'-GTAGAATTCGGATCCATGTCTCCTCCAGTCTAGAT-3' (SEQ
ID NO:19)

Please replace the sequence listing on file in this case with the
attached sequence listing.

Respectfully submitted,



Janet I. Cord
c/o Ladas & Parry
26 West 61st Street
New York, NY 10023
Reg. No. 33,778 (212) 708-1935